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Original Research Article

Isolation of Lignans from The Stem Bark of *Willughbeia coriacea* and Their Cytotoxic Activity

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ABSTRACT

Willughbeia coricea Wall is a herbal medicine to treat diarrhea in Dayak People, Kalimantan, Indonesia. This study aims to determine the structure of the lignans and their anticancer activity against cervical cancer cells (HeLa). Two lignan derivatives, (+)-pinoresinol (**1**) and alyterinate A (**2**), were isolated from the roots of the *W. coricea* Wall. The structures of **1** and **2** were determined based on UV, IR, HRESIMS, 1D (^{1}H and ^{13}C), and 2D NMR (HMQC and HMBC) spectroscopic analysis. Compounds **1-2** showed moderate activity against HeLa cells with an IC_{50} value of 10.1 and 12.5 µM, respectively.

***Keywords*:** *Willughbeia coriacea*, lignan, (+)-pinoresinol, alyterinate A, HeLa cells

Introduction

Willughbeia coricea Wall. (Apocynaceae) is one species of liana plant found in the primary forest of Southeast Asia. This plant was initially cultivated to produce good-quality sap before Brazilian rubber was discovered. A decoction of the leaves and roots of the *W. coriacae* is used to treat diarrhea and a locally consumed edible fruit.[1] Phytochemical data from the *Willughbeia* genus is minimal. *W. cochinchinensis* produces the lignan (pinoresinol, syringaresinol), coumarin (scopoletin, cleomiscosin A), and diarylheptanoid (curcumin, desmethoxycurcumin) derivatives, showing activity as acetylcholinesterase and butyrylcholinesterase inhibitors.[2] *W. coriace* are indigenous plants from Central Kalimantan, Indonesia.[3] Lignans from *W. coricea* stem bark have not been reported for cytotoxic activity. Furthermore, the isolation of compounds **1** and **2** from *W. coricea* stem bark also reported cytotoxic activity against HeLa cells.

Materials and Methods

General experimental procedures
The UV-1900 Shimadzu measured the maximum absorption (λ_{max}), and the FTIR IRAffinity-1S Shimadzu spectrophotometer measured the frequency of the functional groups of lignans. The NMR spectra of the isolated compounds in $CDCl_3$ were measured on a Brucker AVIII 400 spectrometer. The LCT Premier™ XE (Waters) mass spectrometer was used for the determination of chemical formula and mass molecular. Column chromatography (CC) used silica gel and Sephadex LH-20 were used for the isolation of the compounds.

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The melting point of lignans were determined using the Fisher Johns Model 12-144 Apparatus. The visualization of isolated compounds on TLC plate was done using UV lamp and cerium sulfate reagent.

Plant material
The stem bark of *W. coricea* (Plate 1) was collected from Buhut Jaya Village, Central Kapuas, Central Kalimantan, Indonesia, in January 2018. The plant material, encoded specimens (WC-BJCK-AP2), was identified by Dr. Affandi, Departement of Biology, Faculty of Sciences and Technology, Universitas Airlangga, Surabaya, Indonesia.

Extraction and isolation of lignans
The lignans were extracted from dry powder of *W. coricea* stem bark (3.48 kg) using methanol at room temperature for two days. The extract was concentrated using rotavapor to produce a thick methanol extract. The methanol extract was partitioned with hexane and chloroform to produce hexane extracts (26.3 g) and chloroform fraction (12.4 g). The separation of the $CHCl_3$ extract (10 g) by silica gel CC, eluting by hexane-EtOAc (9:1 to 4:1 v/v) afforded two column fractions (A & B). Further purification of column fraction B (1.65 g) on Sephadex LH-20 CC with methanol yielded subfractions B_1 and B_2. The purification of fraction B_2 (615 mg) by silica gel radial chromatography using eluting solvent consisting of hexane-EtOAc (9:1 to 7:3 v/v) afforded compounds **1** (12.8 mg) and **2** (14.2 mg).

Cytotoxic activity
The cytotoxic activity of lignans (**1** and **2**) against cervical cancer cells (HeLa), was conducted using the MTT assay according to the method described by colorimetric method. [4-7] The HeLa cells were cultured in RPMI 1640 medium with 1 mL fetal bovine serum (FBS), 100 µg/mL streptomycin, and 100 µg/mL penicillin added at 37ºC in a 5% CO_2 incubator for 48 hours. Lignans (**1-2)** in the variation concentration were added to the HeLa cells and then incubated for 24 hours at 37 °C with a 5% CO_2 incubator in the 96-well plate respectively. The microplate reader spectrometer measured the active compound's capacity to kill cancer cells at λ = 590 nm. Doxorubicin was used as the cytotoxic assay's positive control.[8-9]

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Plate 1: *W. coricea* Wall.

Result and Discussion

(+)-Pinoresinol (**1**) and alyterinate A (**2**) were isolated from the stem bark of *W. coricea*. Their structures were determined using HR-ESI-MS, UV, IR, 1D, and 2D NMR spectra.

(+)-Pinoresinol (**1**) was isolated as a yellow solid, with melting point of 125-126ºC and having the chemical formula $C_{20}H_{23}O_6$ from the HRESIMS spectrum with a quasimolecular ion $[M+H]^+$ at *m/z* 359.3920 (calcd 359.3916). The UV spectrum of **1** shows the maximum absorption at λ_{max} (log ε): 229 (3.60) and 280 nm (4.00), which are characteristic of the aromatic chromophore. The IR spectrum exhibited absorptions for hydroxy (3468 cm^{-1}), aromatic (1600 and 1518 cm^{-1}), and ether (1238 cm^{-1}) groups, respectively. The ^{1}H-NMR of **1** exhibited two aromatics symmetrical from the ABX system, namely δ_H 6.83 (2H, *dd*, *J* = 2.4; 8.0 Hz, H-6/6'), δ_H 6.88 (2H, *d*, *J* = 8.0 Hz, H-5/5'), and δ_H 6.89 (2H, *d*, *J* = 2.4 Hz, H-2/2'). The ^{1}H NMR also showed two trisubstituted furan rings symmetrical. The trisubstituted furan ring proton consists of an oxy-methine at δ_H 4.73 (2H, *d*, *J* = 4.4 Hz, H-7/7'), a separate oxy-methylene proton at δ_H 4.24 (2H, *dd*, *J* = 4.4 Hz, H-9a/9a´) and δ_H 3.87 (2H, *m*, H-9b/9b´) as well as the methine proton at δ_H 3.10 (2H, *m*, H-8/8´). The methoxy group showed at δ_H 3.90 (6H, *s*, 3/3'-OCH_3). The ^{13}C NMR spectrum of **1** shows perfectly separated ten carbon signals. The carbon signals consist of six aromatic carbons (δ_C 146.8; 145.3; 132.9; 119.0; 114.3; 108.6), three trisubstituted furan ring carbons (δ_C 85.9; 71.7; 54.2) as well as one methoxy carbon signal bound to the aromatic core at δ_C 56.0. The proton and carbon signal of the trisubstituted furan ring should be four signals that indicate it is symmetrical. The two oxy-aryl carbons in the aromatic core (δ_C 146.8; 145.3) indicate that the two oxy-aryls are 1,2-dioxygenated. The 1D NMR spectrum data shows that the isolated aromatic compounds are symmetrical. Based on 1D NMR data, compound **1** is a diepoxy lignan type. The HMBC described the two oxy-aryl carbon's location in the diepoxy lignan skeleton. The methine proton of the furan ring at δ_H 3.10 (H-8/8´) shows a correlation with the oxy-methane carbon (δ_C 85.9) and the aromatic quaternary carbon (δ_C 132.9). The oxy-methane proton signal at δ_H 4.73 (H-7/7´) correlates with the methine carbon (δ_C 54.2), oxy-methylene carbon (δ_C 71.7), two signals of aromatic methine carbon (δ_C 119.0; 108.6) and one aromatic quaternary carbon signal (δ_C 132.9). The location of hydroxy and methoxy groups in the diepoxy lignan skeleton (Fig. 1) was described by correlations of the ABX system of aromatic. The long-range correlations of an aromatic at δ_H 6.88 (H-5/5') to C-7/C-7´, one aromatic methine carbon at δ_C 108.6 (C-2/C-2´), and an oxy-aryl carbon at δ_C 145.3 (C-4/C-4´). The methoxy proton at δ_H 3.90 (3/3'-OCH_3) correlated with the oxy-aryl carbon at δ_C 146.8. Thus, the position of δ_C 145.3 at C-4/C-4´ and δ_C 146.8 at C-3/C-3´. Furthermore, the structure of **1** was identified as (+)-pinoresinol.[9-10]

Alyterinate A (**2**) was obtained as a white solid, showing a melting point of 114-115ºC. The HRESIMS spectrum showed the chemical formula $C_{21}H_{25}O_7$ with a quasimolecular ion $[M+H]^+$ at *m/z* 389.4212 (calcd 389.4210). The ^{1}H-NMR spectrum shows two aromatic proton signals from the ABX system. The three protons are at δ_H 6.76 (1H, *dd*, *J* = 8.2; 1.4 Hz, H-6), δ_H 6.75 (1H, *d*, *J* = 8.2 Hz, H-5), and δ_H 6.93 (1H, *d*, *J* = 1.4 Hz, H-2). Other ABX system protons are seen at δ_H 6.83 (1H, *d*, *J* = 2.0 Hz, H-2'), δ_H 6.73 (1H, *d*, *J* = 8.0 Hz, H-5'), and δ_H 6.64 (1H, *dd*, *J* = 8.0; 2.0 Hz, H-6'). Compound **2** also showed two hydroxy protons [δ_H 7.54 (1H, *s*, 4'-OH), 7.41 (1H, *s*, 4-OH)], and two methoxy groups [δ_H 3.82 (3H, *s*, 3-OCH_3), 3.81 (3H, *s*, 3'-OCH_3)]. The 1H NMR spectrum of **2**, showing a trisubstituted furan ring proton consisting of an oxy-methine at δ_H 5.11 (1H, *d*, *J* = 7.0 Hz, H-7), a discrete oxy-methylene at δ_H 4.02 (1H, *dd*, *J* = 8.5; 6.5 Hz, H-9a') and δ_H 3.70 (1H, *dd*, *J* = 8.5; 7.1 Hz, H-9b'), as well as two methine protons at δ_H 3.05 (1H, *dd*, *J* = 8.8; 7.0 Hz, H-8), and δ_H 2.96 (1H, *m*, H-8´). The methyl proton from the ester is seen at δ_H 3.66 (3H, *s*, $COCH_3$), and the methylene proton signals are separated at δ_H 2.72 (1H, *dd*, *J* = 13.7; 5.4 Hz, H-7a'), and 2.51 (1H, *dd*, *J* = 13.7; 10.3 Hz, H-7b'). The ^{13}C NMR spectrum of **2** shows 19 carbon signals representing 21 carbon signals, indicating that there are two symmetrical carbon atoms, namely two methoxy groups [δ_C 56.1 (3/3'-OCH_3)] and two oxy-aryls [δ_C 145.8 (C-4/4')]. The HMBC spectrum shows the oxy-methine signal at δ_H 5.11 (H-7) correlated with the methine carbon of the furan ring at δ_C 56.8 (C-8), the carbonyl carbon of the ester at δ_C 173.1 (C-9), three aromatic carbons, namely two methine carbons at δ_C 110.1(C-2), δ_C 119.4 (C-6) and a quaternary carbon at δ_C 134.3 (C-1). The carbonyl carbon signal from the ester is reinforced by the correlation between the methyl ester signal at δ_H 3.66, which correlates with δ_C 173.1 (C-9). The signal of the methine at δ_H 3.05 (H-8) correlated to C-1, C-9, an oxy-methine at δ_C 83.3 (C-7), and a methine carbon at δ_C 44.9 (C-8´). The signal at δ_H 2.96 (H-8´) correlated to C-7, C-9, and an oxy-methylene at δ_C 73.4 (C-9´). The long-range correlations of an aromatic proton at δ_H 6.93 (H-2) to C-1, C-6, C-7, and an oxy-aryl at δ_C 146.9 (C-3). The methoxy group at δ_H 3.82 (3-OCH_3) correlated to C-3, indicating the position of the methoxy group bound at C-3. An aromatic signal at δ_H 6.75 (H-5) correlated to C-1, C-3, and an oxy-aryl at δ_C 145.8 (C-4). A hydroxy proton at δ_H 7.41 (4-OH) correlated with C-4, and a methine carbon at δ_C 115.5 (C-5) strengthened the location of the hydroxy group bound at C-4. In other ABX system, an aromatic proton at δ_H 6.83 (H-2') correlated to C-7', a methine carbon at δ_C 121.8 (C-6'), two oxy-aryls [δ_C 148.2 (C-3'), δ_C 145.8 (C-4']. The methoxy group at δ_H 3.81 (3'-OCH_3) correlated to C-3'. A hydroxy proton at δ_H 7.54 (4'-OH) correlated with C-4', indicating the location of the methoxy at C-3' and hydroxy at C-4'. Further, the structure of **2** was identified as alyterinate A.[11]

The cytotoxic activity of compounds **1-2** against HeLa cells, showing moderate activity with an IC_{50} value of 10.1 and 12,.5 µM, respectively (Tanjung *et al.*, 2021; 2018; 2012; Saputri *et al.*, 2021). (+)-Pinoresinol (**1**) is slightly more active than alyterinate A (**2**). Termination of the furan ring produces alcohol and is followed by oxidation reactions and esterification of (+)-pinoresinol (**1**) to alyterinate A (**2**), reducing cytotoxic activity against HeLa cells.[12-13]

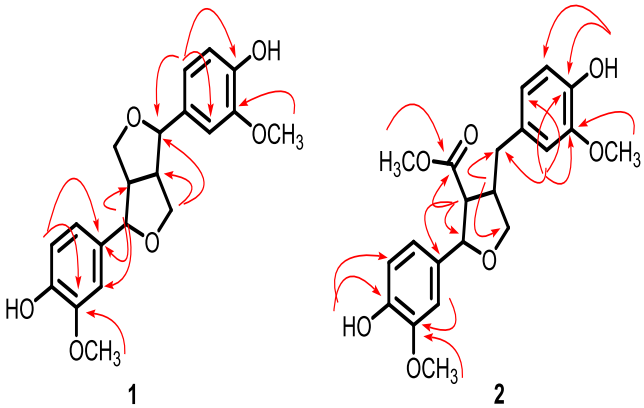

Figure 1: HMBC correlations of **1-2**

Conclusion

Two lignan derivatives, (+)-pinoresinol (**1**) and alyterinate A (**2**), were isolated from the roots of the *W. coricea*. Compounds **1** and **2,** showing moderate activity against HeLa cells.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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